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B-mode and colour Doppler ultrasonography of the milk vein and musculophrenic vein in eight cows during lactation

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ABSTRACT

Eight cows underwent Doppler sonographic examinations of the milk vein and musculophrenic vein from nine days prepartum to 300 days postpartum. A 7.5-MHz linear transducer was used to determine the inner diameter of the veins and blood flow velocities and volumes on the left side in standing, non-sedated animals. Cows were weighed and milk yield measured at all examination times. The milk vein appeared as a vessel with an inner diameter of 2.01 to 2.30 cm immediately beneath the skin. The maximum blood flow velocity ranged from 23.84 to 35.76 cm/s before parturition, increased markedly on the day of calving (day 0), peaked at 61.14 cm/s on day 1 and slowly decreased to 23.84 cm/s by day 300. The profiles of the minimum and mean maximum flow velocities and flow volume were similar. The musculophrenic vein had an inner diameter of 0.76 to 1.07 cm and its distance from the surface of the skin was 1.15 to 1.31 cm. The profiles of the blood flow variables were irregular and did not resemble those of the milk vein. The maximum blood flow velocity ranged from 87.35 to 114.41 cm/s, the minimum velocity from 5.47 to 7.60 cm/s, the mean maximum velocity from 48.55 to 78.74 cm/s and the blood flow volume from 1.02 to 2.44 l/min. The milk vein had a larger diameter (2.16 cm versus 0.90 cm; $P < 0.01$), smaller maximum, minimum and mean maximum blood flow velocities ($P < 0.01$) and a larger blood flow volume than the musculophrenic vein ($P < 0.01$).

Keywords: Cattle; Colour Doppler ultrasonography; Milk vein; Musculophrenic vein; Lactation

1. Introduction

The milk vein (subcutaneous abdominal vein) of dairy cattle is well suited for ultrasonographic examination because it is superficial, large, and does not need to be raised. In addition to scientific value, the vein has considerable clinical importance. Inflammatory changes such as thrombophlebitis, periphlebitis and cellulitis are not uncommon after injections or infusions into the vein or after injuries, and congestion of the milk vein may occur in association with right-sided cardiac insufficiency. The same applies to the musculophrenic vein, albeit to a much lesser degree. The

musculophrenic vein was of minor clinical significance before the era of abdominal ultrasonography, when it was studied in association with the reticulum in cattle (Braun and Götz, 1994). The left and right musculophrenic veins carry blood from the costal parts of the diaphragm to the internal thoracic veins and to the right chamber of the heart via the cranial vena cava. During ultrasonographic examination of the reticulum, the left musculophrenic vein is usually seen as a prominent vessel in longitudinal section. It appears as a hypoechoic structure in the diaphragm a few centimetres from the surface of the skin. (Braun and Götz, 1994; Braun, 2009). The musculophrenic vein is thought to be affected by several conditions including right-sided cardiac insufficiency, compression of the cranial vena cava and myositis of the diaphragm, and detailed investigation of this vessel is therefore warranted (Braun et al., 2009). There is no anatomical link between the milk vein and the musculophrenic vein. There have been a few reports on B-mode and Doppler ultrasonographic examination of the milk vein and musculophrenic vein in cows (Hoegger, 2006; Braun and Hoegger, 2008; Braun et al., 2009) and it was recently shown that blood flow velocities and blood flow volumes in the milk vein are higher in lactating than in dry cows (Braun and Forster, 2012). There was also a trend for an effect of lactation on blood flow variables in the musculophrenic vein. The nature and exact time frame of the changes of blood flow variables in the milk vein and musculophrenic vein during transition from the dry period to lactation, or the nature of these variables during the entire lactation, are not known. The purpose of the present study was to carry out serial B-mode and colour Doppler sonographic examinations of these two veins starting nine days before, until 300 days after calving in eight lactating cows. A secondary goal was to interpret reference values of blood flow variables established previously for these two veins (Hoegger, 2006; Braun and Hoegger, 2008; Braun et al., 2009; Braun and Forster, 2012) in terms of lactational variations.

2. Materials and methods

2.1. Animals

Eight healthy cows, seven Swiss Braunvieh and one Simmental, were used. The cows were from private owners, who provided the cows for the examinations. They were 3.6 to 14.1 years (mean \pm sd = 8.0 ± 3.3 years) old and all were approximately 8.5 months pregnant at the start of the examinations.

2.2. Study design

Each cow was examined 30 times; examinations were carried out daily from nine days prepartum (day -9; day of parturition = day 0) to day 10 after calving and then on days 30, 60, 90, 120, 150, 180, 210, 240 and 300. However, because the calculated and actual due dates usually deviate, examinations were started 14 days before the calculated due date, but only measurements starting on day -9 were used. The examinations were carried out in standing, non-sedated animals between 08.00 and 10.00 hours except on day 0 when they occurred 3 h after calving.

2.3. Clinical examination

The general condition and mental status, appetite, defaecation, urination, rectal temperature, heart and respiratory rates and ruminal and intestinal motility were assessed in the initial physical examination. Simultaneous auscultation and percussion was carried out on both sides of the abdomen, and a urine sample was collected for examination using a test strip (Combur9-Test[®], Roche, Basel). A brief clinical examination, including inspection and palpation of the udder, gross examination of the milk from each quarter and a California mastitis test, was carried out before each ultrasonographic examination. All cows were weighed before and after calving and at each monthly examination.

2.4. B-mode and Doppler sonographic examinations

The procedure for B-mode and Doppler sonography has been described in detail elsewhere (Braun and Hoegger, 2008; Braun et al., 2009; Forster, 2011). The hair over the caudal part of the left milk vein was clipped prior to each examination to yield a 20-cm length and the milk vein was

104 examined in its caudal segment, 10 to 15 cm cranial to the udder. A 50 cm x 50 cm area over the
105 sternal region of the musculophrenic vein was clipped, and the vessel was examined in the sternal
106 area, between the 5th and the 6th intercostal spaces, just ventral to the olecranon. A 7.5 MHz linear
107 transducer (Hitachi Ultrasound scanner EUB 8500) was used to examine the veins. Care was taken
108 not to compress the milk vein during the examination. The veins were first examined in longitudinal
109 and in cross section using B mode, with the transducer surface positioned parallel to the veins. The
110 internal diameters of the veins (i. e., intimal surface-to-intimal surface) were measured electronically
111 in cross section after freezing the ultrasonographic image. Then the distance from the body surface to
112 the intimal surface of the vein was measured. For Doppler recordings, an angulated stand-off was
113 used to achieve an angle of insonation of less than 60 degrees. The colour-Doppler gate was
114 positioned over the vein to visualise blood flow and the cursor line was superimposed and tilted in the
115 direction of blood flow. The sample volume was then positioned in the centre of the milk vein and its
116 size was maximised to cover as much of the venous diameter as possible. Electronic angle correction
117 was applied to achieve adequate alignment of Doppler measurements with blood flow. Subsequently,
118 pulsed-wave Doppler mode was turned on and spectral Doppler tracings were recorded in triplex
119 mode for 10 seconds at each location. Optimal spectral tracings were frozen on the screen. The
120 following variables were automatically measured by the software based on the spectral Doppler
121 profile: Maximum blood flow velocity (peak flow velocity, V_p , highest velocity peak detected during
122 the 10-second recording period), minimum blood flow velocity (V_d , lowest velocity detected during
123 the 10-second recording period), and mean maximum blood flow velocity (average peak velocity,
124 V_m , maximum velocity averaged over the 10-second recording period). The flow volume (FV) was
125 automatically calculated using the time-velocity-integral (TVI) of the intensity-weighted mean blood
126 flow velocity, the cross-sectional area of the vein calculated based on the venous diameter (D)
127 measured in B mode ($CSA = \pi \times (D/2)^2$), and the fractional heart rate (HR) of 6 beats per minute
128 resulting from the given measurement interval of 10 seconds: $FV = TVI \times CSA \times HR$.

129

2.5. Statistical analysis

The software programs StatView 5.1 (SAS Institute, 8602 Wangen, Switzerland) and STATA 10 (StataCorp LP, College Station, Texas, USA, 2009) were used for statistical evaluation. Frequency, mean and standard deviation were calculated for each variable. StatView 5.1 was used for descriptive analysis and regression analysis. The correlations between milk yield, body weight and sonographic variables were calculated using regression analysis. A GLM (General Linear Model) of STATA 10 was used to analyse the profiles of milk yield and blood flow variables. A P value < 0.05 was considered significant.

3. Results

3.1. Clinical findings, body weight and milk yield

With three exceptions, the cows were clinical healthy during the study period. One cow had a uterine torsion, one a left displaced abomasum and one had clinical mastitis, which were all diagnosed promptly and treated successfully. The mean body weight varied from 596 kg to 718 kg (628.2 ± 59.3 kg) during the study period; there was a marked decrease between the start of the study and day 60, which was followed by a plateau and then a mild increase toward the end of the study (Fig. 1). The maximum and minimum mean weights of 717.9 ± 21.3 kg and 595.6 ± 15.1 kg were recorded before calving and at day 60, respectively. The lactation curve peaked at 29.02 ± 2.5 litres on day 8 and then decreased steadily to a minimum of 9.90 ± 6.1 litres on day 300 (Fig. 2).

3.2. B-mode and colour Doppler ultrasonography of the milk vein

The milk vein was visualised in all cows in B-mode and appeared as a vessel with an inner diameter of 2.01 ± 0.41 (mean \pm sd) to 2.30 ± 0.50 cm immediately beneath the skin. The vein was 0.47 ± 0.13 to 0.72 ± 0.39 cm from the body surface. Doppler sonography revealed a spectral display with a band structure of variable width and a wave-like course as described previously (Braun and Hoegger, 2008). The maximum blood flow velocity varied from 23.84 to 61.14 cm/s (Fig. 3). Before

parturition **blood flow** ranged from 28.16 to 35.76 cm/s, and increased **markedly** on the day of calving (day 0) and reached a maximum of 61.14 cm/s on day 1. This was followed by a slow decrease to a minimum value of 23.84 cm/s on day 300. The minimum blood flow velocity varied from 1.75 to 5.65 cm/s (Fig. 4) and had a course similar to the maximum blood flow velocity. It increased from 2.43 cm/sec on day -1 to a maximum value of 5.65 cm/s on day 1. This was followed by a slow decrease to 2.61 cm/s on day 300. The mean maximum blood flow velocity ranged from 18.28 to 42.62 cm/s (Fig. 5), **increasing** from 28.03 cm/s on day -1 to a maximum of 42.62 cm/s on day 1 and then **decreasing** slowly to 14.55 cm/s on day 300. The blood flow volume had a course **similar to that of** blood flow velocities and varied from 1.71 to 4.02 l/min (Fig. 6). Before parturition it ranged from 1.71 to 2.40 l/min, increased to 3.41 l/min on day 1 and reached a maximum of 4.02 l/min on day 2. It decreased slowly thereafter to 1.72 l/min on day 300. Lactation had a significant effect on blood flow volume in the milk vein ($P < 0.05$) and there was a trend for an effect of lactation on the **inner** diameter of the vein ($P = 0.053$).

3.3. B-mode and colour Doppler ultrasonography of the musculophrenic vein

The musculophrenic vein **was** visualised in all cows and appeared as a vessel running parallel to the longitudinal axis of the animal within the diaphragmatic musculature (Braun et al., 2009). The vein was located 1.15 ± 0.2 to 1.31 ± 0.29 cm from the body surface. The **inner** diameter of the vein varied from 0.76 ± 0.23 to 1.07 ± 0.36 cm. The spectral display appeared also as described (Braun et al., 2009) as a broad band with a wave-like course. The profiles of the maximum (Fig. 7), minimum (Fig. 8) and mean maximum blood flow velocities (Fig. 9) and of the blood flow volume (Fig. 10) had irregular variations with no similarities to the profiles of the milk vein. The maximum blood flow velocity varied from 87.35 to 114.41 cm/s, the minimum blood flow velocity from 5.47 to 7.60 cm/s, the mean maximum blood flow velocity from 48.55 to 78.74 cm/s and the blood flow volume from 1.02 to 2.44 l/min. Lactation had no effect on any blood flow variables of the musculophrenic vein.

3.4. Comparison of blood flow variables of the milk vein and musculophrenic vein (Table 1)

During the study period the mean distance of the milk vein from the surface of the skin was smaller than the mean distance of the musculophrenic vein from the surface of the skin (0.58 cm versus 1.20 cm; $P < 0.01$). The mean diameter of the milk vein was larger than that of the musculophrenic vein (2.16 cm versus 0.90 cm; $P < 0.01$). The maximum (99.34 cm/s versus 42.45 cm/s), minimum (6.23 cm/s versus 2.84 cm/s) and mean maximum blood flow velocities (63.20 cm/s versus 27.68 cm/s) of the musculophrenic vein were greater than those of the milk vein ($P < 0.01$). The blood flow volume of the milk vein was greater than that of the musculophrenic vein (2.88 l/min versus 1.52 l/min; $P < 0.01$).

3.5. Correlations between measured variables and body weight and milk yield

There was a significant correlation between blood flow variables of both veins and their internal diameters (milk vein, $r = 0.69$; musculophrenic vein, $r = 0.68$; both $P < 0.05$), but not with body weight. Milk yield did not correlate significantly with the measured variables of the two veins. The profiles of milk yield and the blood flow variables of the milk vein differed significantly ($P < 0.05$); the maximum values for the blood flow variables occurred on days 1 or 2 whereas the lactation curve peaked on day 8.

4. Discussion

Colour Doppler ultrasonographic examinations of the milk vein and musculophrenic veins have previously been performed (Braun and Hoegger, 2008; Braun et al., 2009; Braun and Forster, 2012). Two of these studies used 29 Swiss Braunvieh cows and the same ultrasound machine as in the present study, but a different transducer (Braun and Hoegger, 2008; Braun et al., 2009). However, direct comparison between those studies and the present one is difficult because milk yield was not considered in the former and the sites of the measurements were not exactly the same.

The maximum (42.45 ± 16.60 cm/s versus 45.40 ± 12.5 cm/s) and mean maximum blood flow velocities (27.68 ± 12.4 cm/s versus 33.45 ± 9.5 cm/s) calculated in the present study were very similar to the values obtained previously (Braun and Hoegger, 2008). Studies in dry cows and cows with a daily milk yield of 10 and 20 litres showed that blood flow velocity and blood flow volume of the milk vein are significantly greater in lactating cows than in dry cows (Braun and Forster, 2012). The present study confirmed this and in addition identified the peripartal period as the time when the effect of lactation is most apparent; both blood flow velocities and blood flow volume increased markedly, and the latter peaked two days after calving. The effect of lactation on the inner diameter of the milk vein was not significant ($P = 0.053$) but the profile had a course similar to the flow volume with a peak one day after calving. The formula used to calculate the flow volume in a cylindrical tube ($\text{flow volume} = \pi \times [\text{diameter}/2]^2 \times \text{mean velocity}/2$) illustrates that the variables vein inner diameter, flow velocity and flow volume are related to each other. Thus, an increase in vein diameter or flow velocity, or both, is required to increase the blood flow volume at parturition. Because there was no significant effect of lactation on the diameter of the vein, the increase in flow volume was caused by an increase in flow velocity. The profiles of the different flow velocities were similar in shape with an increase at calving, a peak one or two days later and a steady decrease until day 300. These profiles differed from the lactation curve, which had a slower increase and peaked on day 8. This difference is the most likely reason for the lack of a significant correlation between milk yield and blood flow volume. A peak in the lactation curve at day 8 is unusual because this typically occurs 1.5 to 2 months after calving (Gürtler and Schweigert, 2005). The observation that blood flow volume increased significantly on the day of calving, long before peak lactation occurred, strongly suggests that mammary blood flow is subject to multiple factors (Wendt et al., 1994). The relatively faster decline in blood flow volume compared with decline in milk yield indicates that the mammary gland adapts to increased metabolic demands caused by milk production to ensure adequate availability of metabolites for milk synthesis (Thivierge et al., 2002). Adaptation of the mammary gland to increased milk production has also been described in goats (Nielsen et al., 1990). Doppler

ultrasonography of the milk vein showed that goats with high daily production (3.6 litres) had higher blood flow volumes than goats with lower production (1.9 litres), but the increase was not proportional to the increased production. However, these studies were conducted during lactation and did not include the peripartal period in goats. To our knowledge, there have been no studies describing mammary blood flow during the peripartal period. In an older study using the antipyrin-absorption method, an increase in mammary blood flow after calving was observed in cows, but the increase was not discussed in relation to milk yield (Kjaersgaard, 1968).

In a recent colour Doppler ultrasonographic study of the musculophrenic vein in lactating and dry cows, the mean maximum blood flow velocity was significantly **higher** in lactating cows than in dry cows (Braun and Forster, 2012). This was in contrast to the present study, in which lactation did not affect the profiles of any blood flow variables of the musculophrenic vein and no significant increase in flow velocity occurred at the time of calving. Based on the anatomical relationship of this vein with the mammary gland, these findings were in agreement with our earlier expectations.

4. Conclusions

Colour Doppler ultrasonographic examinations have shown that the effects of lactation on blood flow variables of the milk vein and musculophrenic vein differ. At the time of calving, blood flow velocities and blood flow volume increase in the milk vein, but not in the musculophrenic vein because the former participates in the removal of blood from the udder. This should be considered in future studies involving the milk vein.

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284 **Legends to figures**

285 Fig. 1: Body weight of eight cows from 9 days prepartum to 300 days postpartum (means \pm standard
286 deviations).

287 Fig. 2: Daily milk yield of eight cows from 9 days prepartum to 300 days postpartum (means \pm
288 standard deviations).

289 Fig. 3: Maximum blood flow velocity in the milk vein of eight cows from 9 days prepartum to 300
290 days postpartum (means \pm standard deviations).

291 Fig. 4: Fig. 3: Minimum blood flow velocity in the milk vein of eight cows from 9 days prepartum to
292 300 days postpartum (means \pm standard deviations).

293 Fig. 5: Mean maximum blood flow velocity in the milk vein of eight cows from 9 days prepartum to
294 300 days postpartum (means \pm standard deviations).

295 Fig. 6: Mean blood flow volume in the milk vein of eight cows from 9 days prepartum to 300 days
296 postpartum (means \pm standard deviations).

297 Fig. 7: Maximum blood flow velocity in the musculophrenic vein of eight cows from 9 days
298 prepartum to 300 days postpartum (means \pm standard deviations).

299 Fig. 8: Minimum blood flow velocity in the musculophrenic vein of eight cows from 9 days
300 prepartum to 300 days postpartum (means \pm standard deviations).

301 Fig. 9: Mean maximum blood flow velocity in the musculophrenic vein of eight cows from 9 days
302 prepartum to 300 days postpartum (means \pm standard deviations).

303 Fig. 10: Mean blood flow volume in the musculophrenic vein of eight cows from 9 days prepartum to
304 300 days postpartum (means \pm standard deviations).

1 **Table 1**

2 Comparison of Doppler ultrasonographic variables of the milk vein and musculophrenic vein
 3 (means, standard deviations and ranges of the entire study period. Each value represents the
 4 result of 30 measurements in 8 cows)

Variable	Milk vein	Musculophrenic vein	Difference
Distance between vein and surface of skin (cm)	0.58 ± 0.20 (0.28 – 1.54)	1.20 ± 0.30 (0.66 – 1.94)	P < 0.01
Diameter of vein (cm)	2.16 ± 0.50 (1.46 – 3.32)	0.90 ± 0.30 (0.36 – 1.71)	P < 0.01
Maximum blood flow velocity (cm/s)	42.45 ± 16.60 (9.20 – 84.33)	99.34 ± 29.60 (24.78 – 209.50)	P < 0.01
Minimum blood flow velocity (cm/s)	2.84 ± 1.90 (0.30 – 17.78)	6.23 ± 2.70 (1.28 – 25.35)	P < 0.01
Mean maximum blood flow velocity (cm/s)	27.68 ± 12.40 (12.58 – 69.80)	63.20 ± 20.60 (4.48 – 143.25)	P < 0.01
Blood flow volume (l/min)	2.88 ± 1.20 (0.92 – 8.20)	1.52 ± 1.00 (0.08 – 5.61)	P < 0.01



















